Abnormal dark adaptation kinetics in autosomal dominant sector retinitis pigmentosa due to rod opsin mutation

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Abstract

The time course of dark adaptation was measured in 10 subjects from three families with autosomal dominant sector retinitis pigmentosa (RP) due to mutations in the first exon of the rod opsin gene. In each subject cone adaptation and the early part of the recovery of rod sensitivity followed the normal time course, but the later phase of rod adaptation was markedly prolonged. The recovery of rod sensitivity is much slower than that reported in any other outer retinal dystrophy. Using a model based upon primate data of rod outer segment length and turnover, we have calculated that the delayed phase of the recovery of rod sensitivity in the RP patients tested following strong light adaptation could be due in part to formation of new disc membrane with its normal concentration of rhodopsin rather than in situ regeneration of photopigment.

(*Br J Ophthalmol* 1992; 76: 465–469)

Research into disease mechanisms in retinitis pigmentosa (RP) is complicated by heterogeneity within the disorder. RP may be inherited as an autosomal dominant, autosomal recessive, or X-linked disorder, and there is good evidence of heterogeneity within genetic subtypes.[1-13] Further subdivision of autosomal dominant RP (ADRP) has been achieved by analysis of functional deficits. Two broad categories of RP have been identified which are designated as class I or 'diffuse' and class II or 'regional' forms,[1-4] and the functional characteristics appear to be consistent within families indicating that the functional differences reflect genetic heterogeneity. An additional variant, sector RP is characterised by retinal atrophy seen in only one part of the fundus, usually the lower nasal quadrant, and gross field loss confined to the area of visual field corresponding to the involved retina. The rod and cone electroretinographs show mild reduction in amplitude with normal cone implicit times.[5-7] This pattern of disease is seen in all affected family members, irrespective of age suggesting that the disease, in contrast to other forms of ADRP, is non-progressive or progresses very slowly.

Recent genetic studies have provided evidence for further heterogeneity in AD and X-linked RP.[8-12] About 25% of families with ADRP show mutations of the rod opsin gene on chromosome 3,[13 14] and among this group of patients different patterns of retinal dysfunction may be seen with different mutations.[15-20] Recently it has been shown that genes other than that for rod opsin may transmit ADRP.[21 24]

Here we report the clinical, electroretinographic, and psychophysical findings in 10 patients from three families with autosomal dominant sector RP due to mutations in the rod opsin gene. In each of these families there was markedly slowed rod adaptation.

Patients and methods

Ten affected individuals from three families diagnosed as having sector ADRP were studied (Table 1). Informed consent was obtained after the nature of the procedure had been fully explained. In each patient ophthalmoscopy revealed pigment epithelial atrophy and pigment migration into the lower retina which was most marked in the inferior nasal quadrant. The upper retina had a normal appearance. In each case Goldmann perimetry revealed upper field loss corresponding to the ophthalmoscopically abnormal retina (Fig 1). Electroretinography was performed in nine subjects in accordance with a standard protocol[25] (Table 2). Dark adapted perimetry was performed on the right eye of each subject using red (dominant wavelength 660 nm, subtending 0·9°) and green (dominant wavelength 530 nm, subtending 0·9°) targets. The pupil was dilated with 1% cyclopentolate and the eye dark adapted for 40 minutes before starting the test. At least 17 points at different retinal locations in both upper and lower fields were tested in each case. The apparatus and method for the dark adapted static perimetry have been described previously.[26] Dark adaptation curves were obtained from threshold measurements determined using the green (530 nm, 0·9°) target of the static perimeter or the Tubinger perimeter (dominant wavelength 500 nm; stimulus size 1·7°). We chose to determine the dark adaptation curves at points in the clinically uninvolved retina where the rod threshold elevations were less marked (Table 1). Threshold measurements were made on the right eye. The pupil was dilated, the eye dark adapted for 60 minutes, and a dark adapted threshold was then determined. The area of retina to be tested was then fully light adapted (7·5 log scotopic trolands for 3 seconds) and measurements were continued in the dark for at least 60 minutes (or longer if the patient could tolerate it).

Blood samples were collected from each subject and DNA extracted. Exon sequences were amplified using the polymerase chain reaction (PCR) and screened for mutations using the heteroduplex assay as described pre-

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Accepted for publication 21 February 1992

Table 1 Pre-bleach thresholds and elevation of threshold after 60 minutes of dark adaptation following strong light adaptation. In families 1038 and 1084 the mutation is Gly106Arg, and in 1935 it is Thr58Arg

Subject	RP family	Age	Test location	DA threshold elevation	Additional threshold elevation*
1	1038	36	180M 25E	0·8	1·0
2	1038	64	330M 25E	1·1	1·2
3	1038	74	315M 20E	1·9*	1·1*
4	1084	26	180M 25E	0·9	1·6
5	1084	47	345M 25E	1·1	1·0
6	1935	45	315M 40E	0·6*	0·8*
			315M 50E	0·5*	0·4*
7	1935	18	180M 25E	0·1	1·9
			180M 40E	0·0	1·4
8	1935	16	180M 40E	1·1	1·6
9	1935	21	315M 30E	0·0	1·3
10	1935	50	315M 40E	1·5*	1·4*
			315M 50E	1·5*	2·1*

*Additional threshold elevation = the elevation over the dark adapted threshold after 60 minutes of adaptation following bleach.
DA = dark adapted; M = meridian; E = eccentricity measured in degrees. Thresholds were measured with a LED adaptometer*, or Tubinger perimeter.

Table 2 Amplitudes and implicit times of the electroretinograms

Subject Eye	Age	Dark adapted blue b amp	b imp	Bright flash white a amp	a imp	b amp	b imp	Flicker (30 Hz) b amp	b imp
1 R	36	220	60	160	24	360	50	35	30
L		200	60	120	24	340	50	40	30
3 R	74	200	80	140	26	260	64	10	40
L		180	80	100	27	200	56	15	40
4 R	26	280	60	200	13	420	60	50	30
L		240	60	160	13	400	60	50	30
5 R	47	320	60	160	20	300	50	50	30
6 R	45	110	62	80	22	140	51	20	32
L		115	70	80	22	140	56	20	33
7 R	18	170	74	120	22	280	50	35	30
L		160	74	160	22	280	50	45	30
8 R	16	230	68	140	22	280	60	100	30
L		200	68	160	22	320	60	80	30
9 R	21	170	80	50	22	230	50	25	32
L		100	70	60	22	160	50	35	32
10 R	50	100	84	55	24	180	54	12	34
L		80	84	60	24	140	55	11	36
Normal values									
		319	57	264	15	404	50	43	29
(1 SD)		(89)	(6)	(64)	(2·5)	(88)	(3)	(5)	(1·5)

amp = amplitude in μV; imp = implicit time in milliseconds; no data are available on subject 2.

viously.[27 28] Pairs of oligonucleotide primers were synthesised surrounding rod opsin exons to give PCR products in the range of 200–300 bp. The PCR was carried out using a two-stage reaction profile of 94°C for 30 seconds and 60°C for 3 minutes, repeated 30 times. Aliquots of the PCR reactions mixes were then electrophoresed on D-5000 hydrolink gels (AT Biochem, Malvern, PA, USA for around 2000 Vh). Mismatches due to the presence of a heterozygous mutation caused shifted mobility in heteroduplexed molecules, which could then be further characterised by sequencing the appropriate PCR product.

Results

Rod opsin mutations were identified in each family comprising a change of threonine to arginine at codon 58 in RP1935, and glycine to arginine at codon 106 in RP1038 and RP1084. Both mutations can be detected as altered restriction sites in PCR amplified rod opsin exon 1 (Fig 2).

In all subjects Goldmann visual field loss was confined to the upper field, and dark adapted perimetry confirmed that thresholds for both the 530 nm and 660 nm targets were markedly elevated throughout that region (Fig 1). In the lower field, which was normal on Goldmann perimetry, dark adapted perimetry was normal in some areas but mildly abnormal in others

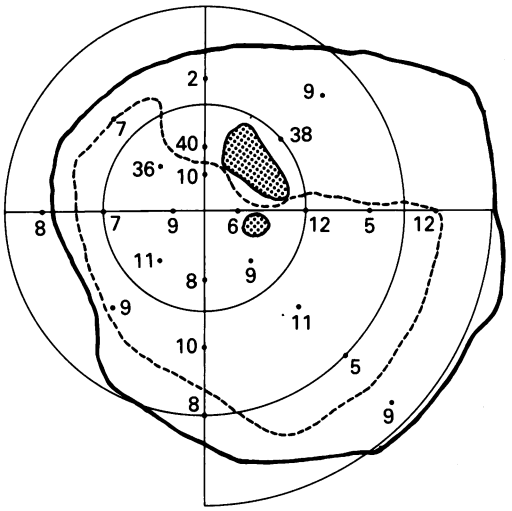

Figure 1 Patient 1: right eye, Goldmann visual fields to IV4e (solid line) and IL4e (broken line) targets. Numerical values indicate dark adapted static perimetry threshold elevation in tenths of a log unit to a green (530 nm) target).

consistent with the regional pattern of disease. This pattern of threshold elevation was a consistent finding in all 10 subjects.

All subjects had normal or mildly reduced rod and cone b-wave amplitudes of the ERG (Table 2). There was a trend for the amplitudes to become less with age but they did not approach the levels seen in non-sector RP. Rod b-wave implicit times were abnormal in all five members of RP1935 and the oldest member of RP1038. Cone implicit times were normal in at least one member of each family.

Dark adaptation was markedly prolonged in all 10 subjects, and none reached their pre-bleach threshold by 60 minutes. At this time the threshold elevation relative to the pre-bleach values ranged from 0·4 to 2·1 log units (Table 1). Inspection of the dark adaptation curves showed that in each case there was a similar pattern of abnormality; cone adaptation was normal, the rod cone break occurred at the normal time, and the early part of the rod adaptation curve followed an apparently normal time course. Subsequently rod adaptation was markedly slowed with a gradual drift down towards the threshold (Figs 3 and 4).

Because of the difficulty with prolonged testing it is not possible to give the exact time for full recovery of rod function. However two subjects agreed to be tested again during the following days. At the end of the initial dark adaptation determinations the subjects returned to normal ambient lighting conditions, and were later retested at the same retinal location at periods ranging from 24 to 120 hours post-bleach. On each occasion the subjects were dark adapted for 1 hour before threshold measurements were taken. Subject 10 showed elevated thresholds of 0·6 and 1·4 logs units above pre-bleach values at two different locations 44 hours after light adaptation, and in subject 7 the threshold was elevated 1·5 log units at 24 hours but had returned to the dark adapted value by 120 hours.

Discussion

Nyctalopia is a prominent and early symptom in

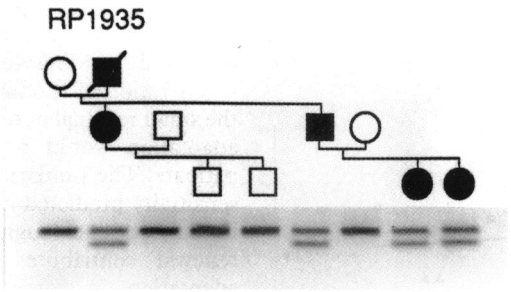

Fig 2A

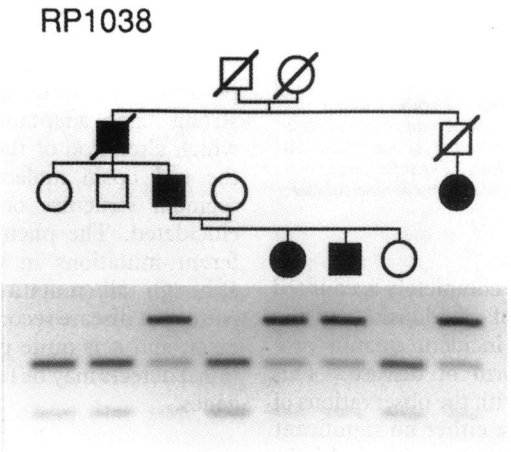

Fig 2B

Figure 2 (A) Family 1935 pedigree, mounted above a photograph of an ethidium bromide stained gel showing PCR amplified exon 1 from members of the family cut with the enzyme Ddel. PCR primers CATGTTTCTG CTGATCGTGC and ACTCTCCCAGACCCC TCCAT give a 295 bp fragment which is normally uncut with the restriction enzyme Dde 1. However the codon 58 ACG to AGG mutation introduces a Dde 1 site, which gives rise to 254 and 41 bp bands. All affected individuals are heterozygous for the uncut and cut fragments. (B) Family 1038 pedigree, mounted above a gel picture showing PCR amplified exon 1 from family members which has been cut with the restriction enzyme Apal. The same PCR primers were used giving a fragment of 295 bp, which is normally cut to 191 and 104 bp fragments by Apa 1. The codon 106 GGG to AGG mutation destroys this site, so that affected individuals are again heterozygous for digested and undigested product.

RP, and elevated absolute thresholds are seen in all types of the disease. Where there is extensive loss of rod function, cones may mediate threshold even under scotopic conditions so that the dark adaptation curve is monophasic. In patients with less advanced disease, most studies have identified a biphasic dark adaptation curve with a normal time course but elevated final threshold.[29 30] However, Alexander and Fishman pointed out that unless a baseline dark adapted threshold is determined before light adaptation it is not possible to be certain that dark adaptation is complete even when thresholds appear to have stabilised.[31] They identified several patients with different types of RP in whom the final thresholds were elevated and the time course of recovery of

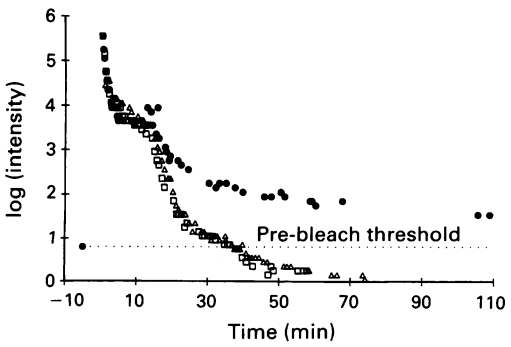

Figure 3 Patient 1: right eye, dark adaptation curve plotted using Tubinger perimeter (500 nm wavelength) at 25 deg eccentricity along the horizontal nasal field meridian. The patient's threshold values (solid circles) and those of two normal observers (open symbols) are plotted relative to normal absolute threshold. The patient's baseline dark adapted threshold is shown as the horizontal line. Adaptation to the 8·0 log scotopic troland seconds light was at time zero.

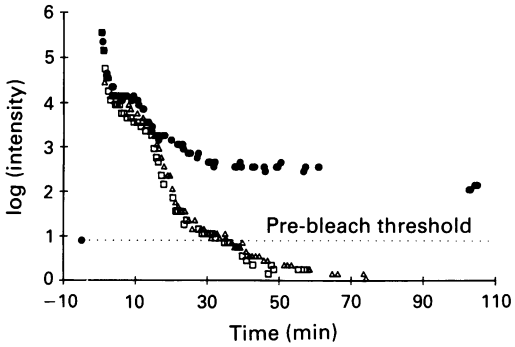

Figure 4 Patient 4: right eye, dark adaptation curve plotted using the Tubinger perimeter (500 nm wavelength) at 25 degree eccentricity along horizontal nasal field meridian. The patient's threshold values (solid circles) and those of two normal observers (open symbols) are plotted relative to normal absolute threshold. The patient's baseline dark adapted threshold is shown as the horizontal line. Adaptation to the 8.0 log scotopic troland seconds light was at time zero.

rod sensitivity was slow. They did not determine that the abnormality was consistently seen within a family or genetic subtype.

Using a similar approach we have been able to show delayed rod dark adaptation in all 10 subjects from three families with autosomal dominant RP. Our patients resemble those designated as sector RP[5-7] in that altitudinal distribution of disease was found consistently in each family whatever the age of the subject. Although the implicit time of the rod and cone b-waves were not normal in all subjects, normal times were found in at least one member of each family.

The functional abnormality in our patients with a mutation at codon 106 is qualitatively similar to that seen with the mutation at codon 58. Slow rod adaptation has been identified previously in some patients with ADRP,[19 20 31 32] including some with altitudinal distribution of disease and known mutations of the rod opsin gene namely: threonine-17-methionine[19]; proline-23-histidine,[20] and threonine-58-arginine.[19] Our results are similar to those previously reported although the slow phase of recovery of rod sensitivity was longer in our patients than in each of the other genotypes. There is no clear explanation for this disparity although the light adaptation protocols were different in that we used more light (8·0 log scotopic troland seconds as opposed to 7·5), and it was delivered in a shorter time (3 seconds as opposed to 60).

There are several possible mechanisms by which abnormality of the rod opsin molecule could result in slowed adaptation. There may be slowed regeneration of rhodopsin as seen in fundus albipunctatus[33 34] or delayed removal of abnormal bleach photoproducts which may interfere with rhodopsin formation or desensitise the rod photoreceptors.[31]

Alternatively, it is possible that abnormal rod opsin could interfere with normal disc assembly, structure, or stability. The prolongation of the recovery of rod sensitivity to a period of several days led us to consider whether or not outer segment renewal may contribute to the later phase of dark adaptation in our patients. In our model we have assumed the dark adapted rod

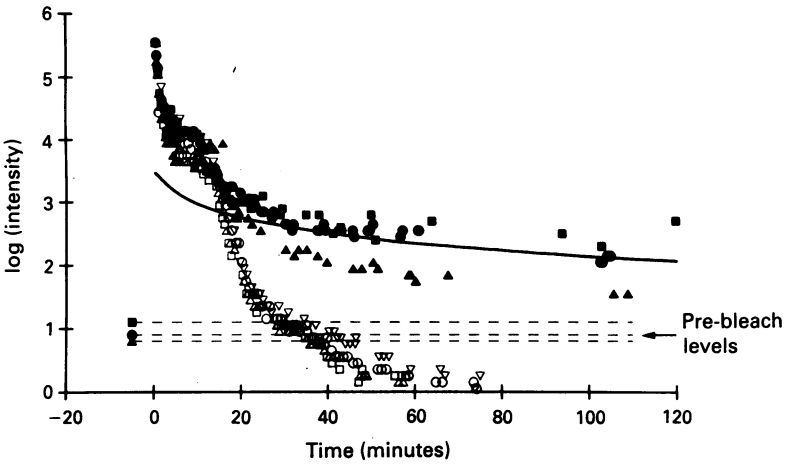

Figure 5 Dark adaptation curves measured under similar conditions to Figures 2 and 3 showing the results from three patients (subjects 1, 4, 8) compared with normal controls (open symbols). These patients are illustrated since dark adaptation was recorded for more than 100 minutes. The pre-bleach thresholds are indicated by the horizontal lines. The solid curve represents the results of the calculation of the effect on threshold of outer segment renewal (see text).

threshold elevation can be completely accounted for by reduced levels of rhodopsin causing decreased absorption of incident quanta proposed in the regional form of disease.[4] This supposition is consistent with the observations of Kemp *et al.*[20] If there were either no significant in situ regeneration of rhodopsin after full light adaptation, or excessive shedding from a shortened outer segment leaving only a greatly truncated functioning outer segment, recovery of rod sensitivity would depend largely on the formation of new outer segment discs with their normal concentration of rhodopsin. In favour of the latter is the suggestion that night blindness in some forms of RP may be caused by progressive shortening of the rod outer segment due to an imbalance between disc shedding and renewal,[35] and the identification of shortened rod outer segments in autosomal dominant RP in a histological study.[36] Using data from primate studies of rod outer segment length and disc turnover,[37] the recovery of rod sensitivity due entirely to regrowth of photoreceptor discs can be calculated. It is possible then to model the effect of different rod outer segment lengths and different rates of renewal on recovery of rod sensitivity. Here we have considered a simple model of an outer segment length of 0·01 μm following intense light adaptation and assumed normal outer segment renewal of 3 μm per day. The predicted recovery of rod sensitivity with formation of new outer segment discs can be calculated using the quantum catch relationship[4] (Fig 5). Using this simple model there is a good fit between the predicted recovery of rod sensitivity and that actually measured in our subjects during the entire abnormal late phase of rod adaptation.

Thus, the very slowed late phase of rod dark adaptation seen in our subjects, may be explained by the regrowth of rod outer segments with their normal concentration of rhodopsin. The data do not distinguish between the two possible mechanisms, namely failure to regenerate rhodopsin on existing outer segment disc membranes and light induced shedding restricting available membrane. However the normal time course of the early phase of recovery of rod sensitivity is more readily explained by the second; if shedding were not complete and in situ regeneration of rhodopsin occurred normally in the small residual outer segment, initial rod dark adaptation would be normal as seen in our patients. The similarity between the recovery of sensitivity predicted by the model and that seen in our patients suggests that outer segment renewal contributes to recovery after light adaptation in some patients. To date this mechanism of recovery from strong light adaptation has received little attention, and is worthy of further study.

The model requires that the outer segments are short as a results of RP and that a major portion of the outer segment is shed following strong light adaptation. The mechanism by which alteration of the amino acid sequence of the rod opsin molecule may influence outer segment structure or stability remains to be elucidated. The phenomenon occurs with different mutations in the rod opsin molecule, although all mutations associated with this pattern of disease recorded to date are on the first exon, and it is quite possible that similar functional defects may be found with defects on other genes.

We are grateful to Professor Barrie Jay and Dr Marcelle Jay for helpful discussion during the preparation of this paper. Thanks also to Brenda Lauffart for expert technical assistance.

This work was supported by the National Pigmentosa Foundation Fighting Blindness USA, British Retinitis Pigmentosa Society, Medical Research Council (UK). The University and the Special trustees of the Royal Victoria Infirmary, Newcastle upon Tyne.

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